Global REACH 2018: The influence of acute and chronic hypoxia on cerebral haemodynamics and related functional outcomes during cold and heat stress

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Short Title: CBF control with thermal and hypoxic stress

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Key Points

- Thermal and hypoxic stress commonly coexist in environmental, occupational and clinical settings, yet how the brain tolerates these multi-stressor environments is unknown
- Core cooling by 1.0 ºC reduced cerebral blood flow (CBF) by 20 – 30% and cerebral oxygen delivery (CDO_2) by 12 – 19% at sea level and high altitude, whereas core heating by 1.5 ºC did not reliably reduce CBF or CDO_2
- Oxygen content in arterial blood was fully restored with acclimatization to 4330 m, but concurrent cold stress reduced CBF and CDO_2
- Gross indices of cognition were not impaired by any combination of thermal and hypoxic stress despite large reductions in CDO_2
- Chronic hypoxia renders the brain susceptible to large reductions in oxygen delivery with concurrent cold stress, which might make monitoring core temperature more important in this context

Abstract

Real-world settings are composed of multiple environmental stressors, yet the majority of research in environmental physiology investigates these stressors in isolation. The brain is central in both behavioural and physiological responses to threatening stimuli and, given its tight metabolic and haemodynamic requirements, is particularly susceptible to environmental stress. We measured cerebral blood flow (CBF, duplex ultrasound), cerebral oxygen delivery (CDO_2), oesophageal temperature, and arterial blood gases during exposure to three commonly experienced environmental stressors – heat, cold and hypoxia – in isolation, and in combination. Twelve healthy male subjects (27±11 years) underwent core cooling by 1.0ºC and core heating by 1.5ºC in randomized order at sea-level; acute hypoxia ($PetO_2$ = 50mmHg) was imposed at baseline and at each thermal extreme. Core cooling and heating protocols were repeated after 16±4 days residing at 4330m to investigate any interactions with high altitude acclimatization. Cold stress decreased CBF by 20–30% and CDO_2 by 12–19% (both $p<0.01$) irrespective of altitude, whereas heating did not reliably change either CBF or CDO_2 (both $p>0.08$). The increases in CBF with acute hypoxia during thermal stress were appropriate to maintain CDO_2 at normothermic, normoxic values. Reaction time was faster and slower by 6-9% with heating and cooling, respectively (both $p<0.01$), but central (brain) processes were not impaired by any combination of environmental stressors. These findings

highlight the powerful influence of core cooling in reducing CDO_2. Despite these large reductions in CDO_2 with cold stress, gross indices of cognition remained stable.

Introduction

In natural settings, whether environmental, occupational or clinical, humans are rarely exposed to physiological stressors in isolation. For example, high altitude mountaineers are exposed to frigid dry air and hypobaric hypoxia (Seys *et al.*, 2013), while athletes competing at moderate altitudes experience both heat and hypoxia (Aldous *et al.*, 2016); sugar cane farmers are chronically exposed to pollution amidst a background of prolonged heat stress and dehydration (Barbosa *et al.*, 2012); and brain injuries such as stroke and traumatic brain injury often present with focal hypoxia and thermal instability (Thompson *et al.*, 2003; Ginsberg & Busto, 2011; Wrotek *et al.*, 2011). Understanding how these stressors interact in health and disease is important, as individual stressors can antagonize (Lloyd *et al.*, 2016), exaggerate (Chu *et al.*, 2007) or additively interact (Lloyd *et al.*, 2015; Lawes *et al.*, 2018); therefore, the nett physiological strain hinges upon these interactions. Yet, there is a paucity of research on how physiological responses change when stressors act in combination. As the brain is central to both physiological and behavioural responses, its ability to tolerate stressful environments dictates whether the human as a whole will tolerate that environment. However, the brain is particularly vulnerable to environmental stress because of its relatively high rate of oxygen consumption and negligible energy reserve. Consequently, the brain requires an uninterrupted supply of blood to sustain this high metabolic rate and remove the resultant heat (Nybo *et al.*, 2002*c*).

Hypoxia, cold and heat each present distinct challenges to the balance of cerebral perfusion, cerebral oxygen delivery (CDO_2) and utilisation. The CDO_2 is determined by the product of cerebral blood flow (CBF) and arterial oxygen content (i.e., $CDO_2 = CBF \times CaO_2$), while utilisation is determined largely by local metabolism (i.e., the cerebral metabolic rate of oxygen, $CMRO_2$). Under normal situations, CDO_2 and $CMRO_2$ are coupled tightly. In acute and chronic hypoxia, changes in CBF compensate for the variations in CaO_2 to maintain CDO_2 (Willie *et al.*, 2014), with little effect on $CMRO_2$ (Severinghaus *et al.*, 1966; Ainslie *et al.*, 2014). Unlike hypoxia, changes in core temperature can differentially and substantially affect not only CBF and CDO_2 but also $CMRO_2$. For example, temperature changes alter metabolic rate via effects on Brownian motion; this can be mathematically illustrated using Svante Arrhenius plots, from which a Q_{10} temperature coefficient is derived (Logan, 2009; Bain *et al.*, 2015). Values derived in deep anaesthesia combined with hypothermia (Stone *et al.*, 1956; MacVeigh *et al.*, 1997) and exercising heat stress (Nybo *et al.*,

2002*a*) indicate a Q_{10} of cerebral tissue in the range of 1.6 – 3, i.e. $CMRO_2$ changes by 7 – 20% per degree Celsius change in brain temperature.

The effect of systemic cold stress on CBF and CDO_2 remains largely unknown, but reductions in CBF during brief bouts of cold water immersion appear to be specific to passive exposure and mediated by thermally-induced hyperventilation (Mantoni *et al.*, 2008). More is known on the effects of heat stress on CBF regulation. During passive and active heating, CBF and $CMRO_2$ become uncoupled as CBF tends to decrease (Nybo & Nielsen, 2001; Nelson *et al.*, 2011), which can be managed only by elevating oxygen extraction (Nybo *et al.*, 2002*a*). The heat-induced reduction in CBF is mediated primarily by arterial hypocapnia secondary to heat-induced hyperventilation; this observation is evidenced by a partial (Brothers *et al.*, 2009) or full (Nelson *et al.*, 2011; Bain *et al.*, 2013) restoration of CBF during heat stress when eucapnia is acutely restored or when hyperventilation is voluntarily supressed (Tsuji *et al.*, 2015, 2019). Functionally, each of these stressors in isolation – hypoxia, cold, and heat – have been shown to impair cognitive function (Simmons *et al.*, 2008; Muller *et al.*, 2012; Paulauskas *et al.*, 2015; Piil *et al.*, 2017). The link between CBF and CDO_2 to cognitive functioning is complex in these environments. Some reports show cognitive impairments with acute and prolonged hypoxia despite global CDO_2 and $CMRO_2$ being maintained, a conundrum that might be partly explained by regional reductions in CBF that become more pronounced with continued hypoxic exposure (Lawley *et al.*, 2017). Severe heat stress causes large decreases in CDO_2 but $CMRO_2$ is maintained or even increased and cognitive impairments are observed only when the task complexity is maximized (Nybo *et al.*, 2002*b*; Trangmar *et al.*, 2015; Piil *et al.*, 2017). Whole body cooling decreases both central and peripheral nerve conduction velocity and has been shown to slow central information processing (Rammsayer *et al.*, 1995). The link between CBF regulation and cognition is of particular relevance in these contexts as cognitive decline in extreme environments poses a significant threat to survival.

It seems entirely unknown which factors mediating CBF prevail when thermal stressors are imposed on acute and chronic hypoxia. Do mechanisms for maintaining CDO_2 stability conflict with those defending thermal balance during combined thermal and hypoxic stress? What is the nett effect and functional consequences of adding a vasodilatory hypoxic stimulus on a potentially vasoconstricting cold- or heat-stressed brain? And, how do the ventilatory (e.g. respiratory alkalosis) and haematological (e.g. haemoconcentration) adaptations to chronic hypoxia alter these mechanistic responses and functional outcomes? These questions remain seemingly unexplored, yet are paramount in understanding how the brain tolerates such real-world multi-stressor environments. The goal of this investigation was therefore to explore the mechanisms that regulate CBF and CDO_2 during cold and heat stress under conditions of acute and chronic hypoxia. Our

secondary aim was to examine how these stressors (in isolation and combination) might impact functional outcomes that are pertinent to survival in extreme environments, i.e. thermal perceptions and cognition. It was hypothesized that: (1) both cold and heat stress would reduce CDO_2 by virtue of decreases in CBF mediated by hyperventilation-induced hypocapnia; (2) the reductions in CBF with cold and heat stress would be restored by acute hypoxia (via vasodilation), but the lower CaO_2 would compromise CDO_2; (3) during acclimatization to high altitude, ventilatory and haematological adaptations would facilitate maintenance of CDO_2 but reductions in CBF with *concurrent* cold and heat stress would compromise CDO_2; and (4) combined chronic hypoxic and cold stress would cause the greatest impairment in cognitive function owing to the greatest decrease in CDO_2.

Methods

Ethical approval

Ethical approval was granted by the Clinical Research Ethics Board at the University of British Columbia (H17-02687 and H18-01404) and by the Institutional Human Ethics Committee at the University of Otago (H18/022), and conformed to the *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from all volunteers prior to participation in the study. The current study was a standalone experiment that was part of the Global REACH (Research Expedition on Altitude-related Chronic Health) expedition to Cerro de Pasco, Peru in July of 2018. As such, volunteers were participant to multiple experimental investigations during both sea level and high altitude testing. Care was taken, however, to ensure that no experimental interventions overlapped.

Experimental Design

Participants were exposed to core cooling by 1.0 ºC and heating by 1.5 ºC with superimposed acute hypoxia at sea level (Kelowna, British Columbia, Canada; 344 m) and after 16 ± 4 days of chronic hypoxia at high altitude (Cerro de Pasco, Peru; 4330 m). At sea level, participants were made acutely hypoxic at baseline core temperature and again after being both passively cooled and heated. This generated three experimental conditions: normoxia, acute normobaric hypoxia and chronic hypobaric hypoxia, at three thermal stages: baseline/normothermia, cold (-1.0 ºC core temperature), and hot (+1.5 ºC core temperature). At each thermal stage, measurements were taken under poikilocapnia and then once end-tidal CO_2 ($PetCO_2$) was restored to baseline/normothermic pressures to isolate the role of arterial CO_2 ($PaCO_2$) on CBF. A schematic of the testing protocol is illustrated in Figure 1, and explained below.

The order of thermal manipulation was randomized at sea level and almost balanced for the seven participants that completed cooling and heating at both altitudes; four started with heating and three with cooling. This order was replicated at high altitude. Participants avoided heavy exercise, caffeine and alcohol for 12 hours preceding testing, and were fasted for at least two-hours. Participants were provided with a hypotonic beverage (20 g/L glucose, 1.7 g/L salt; room temperature) to consume *ad libitum*, but were restricted from drinking for at least 15-minutes prior to CBF measurements. Participants did not take prophylactic medications for altitude illness during the rapid ascent to high altitude (~6 h via car from Lima) and none were experiencing symptoms of altitude illness at the time of high altitude testing.

[Figure 1]

Participants

Twelve healthy male participants (aged 27 ± 11 years, body mass index = 23.7 ± 1.8 kg m^{-2}) were recruited from the expedition; they were normotensive, non-smokers and otherwise healthy with no previous history of cardiovascular or respiratory diseases. Of these 12 participants, one participant only completed sea level testing, and one other only high altitude testing. Three participants completed only heating due to previous afflictions with cold stress and two completed only cooling due to time constraints. In total, there were nine cold and nine heat exposures at sea level, and eight cold and ten heat exposures at high altitude. The number of participants included in each experimental step is presented in the table and figure captions.

Experimental protocols

Following the application of thermistors (see *Thermometry* below), cardiorespiratory devices, and radial artery cannulation, 34 ºC water was circulated through a water-perfused suit (Med-Eng, Ottawa, ON Canada) to maintain a stable core temperature while participants rested supine. Thermal perceptions and baseline cognition were measured after ~5 minutes of quiet rest. Measures of CBF were made during: (1) quiet room air breathing, (2) during voluntary iso-oxic hyperventilation that provoked a drop in $P_{ET}CO_2$ of 10 mm Hg, and (3) acute poikilocapnic hypoxia at 50 mm Hg of the partial pressure of end-tidal oxygen ($P_{ET}O_2$) to simulate the magnitude of hypoxaemia in Cerro de Pasco (4330m above sea-level). Participants were then cooled or heated and baseline measures were repeated, except that thermal stress-induced hypocapnia was restored to normothermic values instead of reduced with hyperventilation.

Passive cooling

Cooling was achieved using cold water immersion to the clavicles in an inflatable pool. The water temperature was matched within participants and ranged between 15.5 and 17.7 ºC. The water was stirred manually throughout immersion. Immersion was terminated when one of three criteria were met: (1) core temperature decreased by 1.5 ºC, (2) two hours of immersion elapsed, or (3) the participant could no longer tolerate the cold and asked to be removed. A matched change of 1.5 ºC core temperature was initially targeted, however, four participants had robust shivering responses that defended such decreases in core temperature within the two hour immersion. In these cases, participants completed 10 – 15 active squats to induce a core temperature after-drop immediately before getting on the assessment bed. One participant reached thermal tolerance before two hours of immersion elapsed. If a drop of 1.5 ºC core temperature was not achieved at sea level, the exact magnitude of cooling was noted and matched during high altitude testing. After cooling, participants were assisted out of the tub and moved to the assessment bed, after which ice water was circulated through the water-perfused suit to maintain core temperature for the duration of the measurements. *Passive heating*

Heating was achieved by circulating 48 – 49 ºC water through the water-perfused suit and covering the participant in wool blankets leaving only the head exposed. Participants remained supine throughout. Measures of CBF, blood gases, cognition and thermal perceptions were taken at +0.5 ºC in an attempt to quantify a dose:response relationship for heat stress and examine the influence of heat-induced hypotension without concurrent hypocapnia. When oesophageal temperature had increased 1.5 ºC, the suit's water temperature was reduced and the blankets were removed to stabilize core temperature. Once all measurements were collected, circulating water temperature was reduced to ~22 ºC to uncouple core and skin temperature and CBF was measured again.

Respiratory gas control

The $P_{ET}O_2$ and $P_{ET}CO_2$ were controlled by a portable dynamic end-tidal forcing system, which has been described in detail elsewhere, and validated for use at high altitude (Tymko *et al.*, 2015). Briefly, the gas control system integrates respiratory volumes and end-tidal gas compositions to prospectively generate inhaled gas compositions that will force end-tidal gas concentrations to a pre-determined target. At sea level only, acute hypoxia was induced by forcing $P_{ET}O_2$ down to 50 mm Hg while $P_{ET}CO_2$ remained uncontrolled, i.e. poikicapnic. The $P_{ET}O_2$ and $P_{ET}CO_2$ achieved during this baseline hypoxic stage were noted, and imposed during both cold and heat stress. When end-tidal gases reached these targets, the participant remained clamped for eight-minutes to ensure CBF

and ventilation ($\dot{V}_E$) stabilized before CBF measurements were acquired. In addition, decreases in $P_{ET}CO_2$ that occurred naturally during heating or cooling were restored to normothermic values for 3 – 4 minutes and CBF was again measured. In doing so, CBF was assessed with nearly exactly matched end-tidal gases (and very closely matched arterial blood gases; see Table 1) during normoxic and acute hypoxic conditions at each thermal stage during sea level testing.

Measurements

Thermometry: Core temperature was measured in the rectum and oesophagus, but the oesophageal index was used as the criterion index. Oesophageal temperature was measured using a T-Type thermocouple probe (RET-1, Physitemp Instruments, Clifton, NJ, USA) inserted to a depth relative to standing height (Mekjavic & Rempel, 1990) and rectal temperature was measured at a depth of ~15 cm using a general purpose sterile thermistor (Mon-A-Therm, Covidien, Mansfield, MA, USA). Skin temperatures were measured every 10 seconds at each of 6 sites: forehead, scapula, forearm, finger, thigh and calf using insulated surface thermistors (Skin Thermistors EUS-U-V5-V1, Grant Instruments, Cambridge, UK) and data were saved on a portable logger (Squirrel v. 2010, Grant Instruments, Cambridge, UK).

Blood gases, oximetry and metabolites: Local anaesthetic (1% lidocaine) was injected above the radial artery before cannulation. The radial artery was visualized under ultrasound guidance and cannulated with a 20-gauge cannula (Arrow, Markham ON, Canada). The cannula was attached in series to a waste-less sampling system (VAMP, Edwards Lifesciences, CA, USA) and pressure transducer levelled to the height of the right atrium for continuous beat-by-beat intra-arterial blood pressure (ADInstruments, Dunedin, NZ). Blood samples were analysed immediately for pH, PaO_2, $PaCO_2$, HCO_3^-, arterial oxygen saturation, osmolality, haematocrit (Hct) and haemoglobin concentration ([Hb]; ABL90 FLEX, Radiometer, Copenhagen, Denmark). Blood viscosity was simultaneously measured at each stage using a cone and plate viscometer (DV2T Viscometer, Brookfield Amtek, MA, USA). Viscosity measurements were acquired in duplicate at a shear rate of 225 s^{-1} at the participant's current oesophageal temperature during each stage. For the present study, the coefficient of variation of measurement for the arterial blood gas and viscosity samples was <3%. Arterial blood gas measurements were temperature-corrected to the oesophageal temperature at the time the sample was taken, using previously derived constants and logarithmic equations (Severinghaus, 1966).

Cardiorespiratory: Electrocardiogram and intra-radial blood pressure were sampled at 1 kHz and a beat-by-beat average of heart rate (HR) and arterial pressure (MAP) were recorded. Breathing frequency (f_B), tidal volume (V_T), $\dot{V}_E$ and partial pressures of O_2 and CO_2 were similarly sampled at 1 kHz using an analog-to-digital data acquisition system (PowerLab/16SP, ADInstruments, Dunedin, New Zealand). The $\dot{V}_E$ and expired O_2 and CO_2 fractions were used to calculate the rate of oxygen uptake ($\dot{V}O_2$). Echocardiographic assessments were performed with participants resting in the left lateral decubitus position, by the same sonographer (T.G.D.), using a portable ultrasound system (Vivid Q, GE Healthcare, Piscataway, NJ, USA). The integral of left ventricular outflow velocity and the area of aortic annulus were calculated to provide a measure of cardiac stroke volume, which was used for the calculation of cardiac output ($\dot{Q}$).

Cerebral blood flow: Simultaneous blood velocity and vessel diameter measurements were obtained in the right internal carotid artery (ICA) and external carotid artery (ECA), and left vertebral artery (VA) using a portable ultrasound system (Terason uSmart 3300, Burlington, MA, USA). The ICA and VA were insonated concurrently by two sonographers on opposite sides of the participant and the ECA was insonated immediately thereafter. The ICA velocity and diameter were captured > 2 cm from the bifurcation and care was taken to avoid turbulent flow profiles and tapering of vessel diameter. ECA velocity and diameter were captured > 1 cm from the bifurcation and areas with dense branching were avoided. The VA was captured between C4 and C5 or C5 and C6. Locations for all CBF measurements were replicated within participants as much as possible. Captured videos were saved and stored for offline analysis using commercially available automated edge-detection software (Cardiovascular Suite v3.5, QUIPU, Pisa, Italy). The between day coefficient of variation for ICA diameter and velocity were 1 and 7%, respectively, and 2 and 11% for ECA (T.D.G.). Scanning of the VA was shared between three experienced sonographers (A.P., R.L.H. and T.D.G.) and all were supervised by one investigator (T.D.G.) to ensure consistency between scanners. Mean blood flow velocity was calculated as the product of half the peak envelope velocity and vessel cross-sectional area. Blood flow measures were averaged over the duration of the video (~1 min per artery). Due to excessive movement caused by high rates of $\dot{V}_E$ and vigorous shivering, two videos from a single participant did not provide sufficient quality for reliable blood flow measures in the VA and ECA during cold stress at sea level.

Cognition and thermal perceptions: Pro-point and anti-point tasks were used as an index of cognitive function. Briefly, pro-point tasks measure reaction time to a visual on-screen stimulus and provide an index of stimulus-driven visuomotor function. Anti-point tasks incorporate the additional task requirement of inhibiting the immediate reflexive response. Combining both components (pro-

point/anti-point) provides an assessment of visuomotor and cognitive control and the difference of combined pro-point/anti-point and pro-point reaction time eliminates the influence of nerve conduction velocity shifts caused by changes in temperature. Within our lab (n=25) this specific cognitive battery (and the variables we analysed) has been shown to have good-to-excellent test-retest reliability both within and between days [within day intraclass correlation coefficient = 0.92 and between day = 0.82; and within day coefficient variation = 2.8% and between day = 3.9%], and was selected based this merit and quick time to completion (~3 min). Each participant was instructed how to perform the cognitive battery and completed one familiarization test immediately prior to testing at sea level and high altitude. Thermal perceptions (ranging from 1 = unbearably cold to 13 = unbearably hot), thermal discomfort (ranging from 1 = comfortable to 9 = extremely uncomfortable) and feeling state (ranging from -5 = very bad to +5 = very good) were assessed at each thermal stage.

Calculations

All thermometry and cardiorespiratory measures (with exception of echocardiography) were averaged over the period in which CBF measurements were being made, amounting to 3 – 5 minute bins (LabChart v.8, ADInstruments). Mean skin temperature was calculated as:

$$\bar{T}sk = (0.35 * T_{Scapula}) + (0.20 * T_{Forearm}) + (0.35 * T_{Thigh}) + (0.10 * T_{Face}). \quad \text{Eq 1:}$$

In some cases T_{Calf} was used in place of T_{Thigh} due to unreliable thermocouples. The calculation of $\bar{T}sk$ was always matched within participants at sea level and high altitude.

CBF was calculated as:

$$CBF\ (ml\ min^{-1}) = (2 \cdot \dot{Q}_{ICA}) + (2 \cdot \dot{Q}_{VA}), \quad \text{Eq 2:}$$

where $\dot{Q}_{ICA}$ represents volumetric flow through the right ICA and $\dot{Q}_{VA}$ for the left VA. This formula assumes blood flow between ICA's and VA's is equal. Cerebrovascular conductance (CVC) was calculated as the quotient of CBF and MAP:

$$CVC\ (mL\ min^{-1}\ mm\ Hg^{-1}) = CBF / MAP\ (mm\ Hg), \quad \text{Eq 3:}$$

and cerebrovascular reactivity (CVR) was calculated as the quotient of CBF and either arterial oxygen saturation (SaO_2) or $PaCO_2$:

$$CVRO_2\ (mL\ min^{-1}\ \%O_2^{-1}) = CBF / SaO_2\ (\%); \quad \text{Eq 4:}$$

$$CVRCO_2\ (mL\ min^{-1}\ mm\ Hg^{-1}) = CBF / PaCO_2\ (mm\ Hg). \quad \text{Eq 5:}$$

Arterial oxygen content (CaO_2) was calculated with measures of SaO_2, [Hb] and PaO_2 using the formula:

$$CaO_2\ (\text{mL dL}^{-1}) = ([\text{Hb}] \cdot 1.36 \cdot \frac{SaO_2}{100}) + (0.003 \cdot PaO_2) , \quad \text{Eq 6:}$$

where [Hb] is the concentration of haemoglobin, 1.36 is the affinity of O_2 to haemoglobin, SaO_2 is the percentage of haemoglobin saturated with oxygen, 0.003 is the fraction of free O_2 dissolved in the blood. The product of CBF and CaO_2 was used to calculate CDO_2:

$$CDO_2\ (\text{mL } O_2\ \text{min}^{-1}) = CBF \cdot CaO_2 / 100. \quad \text{Eq 6:}$$

Statistical analysis

Variables were individually analysed longitudinally using linear mixed-effect model analysis. The oxygen status (normoxia, acute, and chronic hypoxia), and thermal status (normothermia, cold, and hot) were modelled as fixed effects, and participants (and associated interactions) were modelled as a random effect (where appropriate, see below). Due to a theoretically plausible effect of order (i.e., systematically different response in those going from cold to hot vs. those going from hot to cold), order of completion was accounted for statistically. Homogeneity of variances was assessed visually via plotting of residuals versus model-fitted values and formally with Levene's test across all combinations of factors in the model. Linearity and approximate normal distribution of residuals were assessed via visual inspection of histograms and Q-Q plots of model and individual residuals and formally with Shapiro-Wilk test. Approximate normal distribution of random effects was assessed via visual inspection of Q-Q plots. Akaike's Information Criteria and model parsimony were used to determine variance/covariance structure of model errors, random and fixed effect structure, and model inclusion. Multiple comparisons were made using the estimated marginal means (derived from the linear mixed models) via the Tukey methods. Mixed model analysis (packagesL 'lne4' and 'emmeans') was performed using R (R Development Core Team, 2008) and figures were generated using Prism (GraphPad Prism 8.1.0, 2019) and Inkscape (Inkscape 0.92.4, 2017). Descriptive statistics in text are reported as raw means ± SD, whereas comparisons of interest are reported as estimated marginal means with corresponding 95% confidence limits [lower limit, upper limit]. To aid in interpretation, main effects (and any associated interactions) are provided in figures, and (where appropriate) post-hoc p-values are presented.

Results

Effectiveness of interventions (Figure 2)

Core temperature displacements were similar between sea level and high altitude; being increased by 1.5 ± 0.1 and 1.6 ± 0.3 ºC, respectively, and decreased by 1.0 ± 0.5 and 0.9 ± 0.5 ºC (Hypoxia main effect, p=0.33). Acute hypoxia at sea level resulted in a SaO_2 of 82-85% across the different thermal states (p<0.01). Normothermic $P_{ET}O_2$ was consistent between acute and chronic hypoxia (50 [49, 50] and 51 [47, 55] mm Hg, respectively, p=0.64), but SaO_2 was higher at high altitude when compared to acute hypoxia at sea level, ranging from 87-90% across the thermal stages (p<0.01). The duration of heating was 1 hour and 22 min at sea level (± 20 min) and high altitude (± 41 min), while the duration of cooling was 1 hour and 45 min at sea level (± 18 min) and 1 hour and 12 minutes at high altitude (± 31 min). The rate of oesophageal heating was the same at sea level and high altitude (p=0.55); however, the rate of cooling tended to be faster at high altitude (p=0.06, Figure 2).

[Figure 2]

Thermoregulatory and cardiovascular responses with combined thermal and hypoxic stress (Table 1)

Despite a matched increase in core temperature, $\bar{T}sk$ increased more with heating at high altitude than at sea level (Heat – High altitude interaction, p<0.01). Moreover, $\bar{T}sk$ decreased more during cooling at high altitude than when acutely hypoxic at sea level (Cold – High altitude interaction, p<0.01), but was not different from normoxic conditions at sea level (interaction, p=0.43). Facial skin temperature was 0.5 ^{o}C [0.0, 1.1] lower at high altitude when compared to sea level, irrespective of thermal stress (High altitude altitude main effect, p=0.03).

Core heating doubled HR whether in normoxia, acute hypoxia, or chronic hypoxia (Heat main effect, p<0.01). Core cooling increased HR by 16 bpm [10, 22] regardless of normoxia or hypoxia (Cold main effect, p<0.01). Acute hypoxia increased HR by 16 bpm ([10, 22], p<0.01), and remained elevated during acclimatization to high altitude (Acute hypoxia – Chronic hypoxia main effect, p=0.20). Core heating increased $\dot{Q}$ by 2.7 l min^{-1} ([2.2, 3.3]; main effect, p<0.01), and core cooling increased $\dot{Q}$ by 1.1 l min^{-1} [0.5, 1.7], both irrespective of hypoxia (Cold main effect, p<0.01). Baseline $\dot{Q}$ was significantly lower at high altitude when compared to acute hypoxia at sea level (Acute hypoxia – Chronic hypoxia main effect, p=0.01), and neither were reliably different from normoxia (p>0.14). The MAP was unaffected by acute or chronic hypoxia (Hypoxia main effect, p=0.35), nor was there an interaction effect between thermal state and hypoxia (p=0.99). Heating caused a 15

mm Hg [-10, -19] reduction in MAP ($p<0.01$), whereas cooling caused a 16 mm Hg [+11, +21] increase in MAP ($p<0.01$).

Core heating and acute poikilocapnic hypoxia each increased $\dot{V}_E$ by 3–4 l min^{-1} when imposed in isolation (main effects, $p<0.01$), whereas when imposed concurrently they increased $\dot{V}_E$ by 39 L min^{-1} ([27, 46]; Heat – Acute hypoxia interaction, $p=0.01$). Core cooling also potentiated the effect of hypoxia; the increase in $\dot{V}_E$ with cooling was 20 l min^{-1} [10, 30] when normoxic, and 36 l min^{-1} [27, 46] when combined with acute hypoxia, which remained similarly elevated after acclimatization to hypoxia (Cold – Hypoxia interaction, $p=0.01$). Consequently, $PaCO_2$ was differentially affected by thermal and hypoxic stress (Thermal – Hypoxia interaction, $p<0.01$). The arterial hypocapnic response with core heating by 1.5 ºC was similarly modest at both sea level and high altitude (-3 mm Hg [0, 6]), whereas the hypocapnia induced by cooling was slightly greater (-8 mm Hg [5, 11] at sea level and -3 mm Hg [0, 7] at high altitude). Arterial HCO_3^- concentration was stable with core heating at sea level and high altitude. In contrast, HCO_3^- was decreased by core cooling to a similar extent at sea level and high altitude; however, this decrease was smaller when acutely hypoxic at sea level (Cold – Acute hypoxia interaction, $p=0.03$). Acclimatization to hypoxia decreased HCO_3^- by 6 meq l^{-1} [-4, -7], $p<0.01$).

After ~16 days at high altitude Hct had increased by 5.9% ([5.2, 6.6]; High altitude main effect, $p<0.01$). Cooling increased Hct similarly by 5.4% [4.9, 6.0], whereas core heating increased Hct by 3.1% [2.5, 3.8], both independent of altitude (both $p<0.01$). Changes in blood viscosity closely followed those of Hct.

Table 1. Summary of thermal, cardiovascular, metabolic, cerebrovascular, haematological, perceptual and cognitive data at each thermal and hypoxic stage.

	Baseline			**+0.5 ºC**		**+1.5 ºC**			**-1.0 ºC**			**Main effect**		**Interaction**
	NX	AHX	CHX	NX	CHX	NX	AHX	CHX	NX	AHX	CHX	Thermal	Hypoxia	
Thermometry (º C)														
T_{Oes}	36.8±0.3	36.8±0.3	36.9±0.4	37.4±0.3	37.5±0.4	38.3±0.3	38.2±0.3	38.5±0.4	35.9±0.7	35.9±0.8	35.8±0.7	<0.01	0.33	0.83
T_{Rec}	36.7±0.3	36.6±0.2	36.7±0.3	36.9±0.2	37.0±0.4	37.8±0.2	38.0±0.2	38.1±0.5	35.7±0.7	35.9±0.7	36.2±0.7	<0.01	0.06	0.36
T_{Face}	33.3±0.9	33.3±1.1	32.1±0.9	33.8±1.3	33.3±1.3	35.4±1.4	35.1±1.7	35.4±0.9	31.2±0.9	31.3±0.8	30.7±0.9	<0.01	<0.01	0.09
$\bar{T}_{Skin}$	34.6±0.5	34.7±0.5	33.8±1.1	37.1±0.6	37.4±0.7	37.8±0.6	37.7±0.5	38.2±0.5	24.9±2.9	26.7±2.8	23.6±2.8	<0.01	0.83	**<0.01**
Cardiovascular														
HR (bpm)	54±8	65±11	66±12	80±12	96±12	107±16	128±25	124±17	69±14	84±19	77±11	<0.01	<0.01	0.28
MAP (mm Hg)	93±7	94±8	97±8	83±5	86±6	82±7	84±10	83±8	107±12	107±13	112±10	<0.01	0.35	0.99
$\dot{Q}$ (l min^{-1})	4.9±0.8	5.7±1.0	4.8±0.8			8.3±1.3	8.4±1.1	6.9±0.4	6.4±1.4	6.1±1.4	6.0±1.2	<0.01	<0.01	0.10
TPR(mm Hg min l^{-1})	19.5±3.3	16.8±3.6	20.3±3.3			10.3±1.9	10.4±1.4	12.3±1.3	18.3±5.9	18.7±6.0	19.2±3.2	<0.01	<0.01	0.37
Metabolic														
fB (bpm)	14±	17±5	16±2			16±5	31±10	24±11	21±6	24±5	24±5	<0.01	<0.01	0.11
V_T (L)	0.9±0.2	0.9±0.3	1.0±0.2			1.0±0.3	1.6±0.5	1.4±0.2	1.6±0.5	2.1±0.4	2.2±0.5	<0.01	<0.01	**<0.01**
$\dot{V}_E$ (l min^{-1})	11±2	14±3	16±3			15±2	50±25	32±16	31±5	50±9	51±19	<0.01	<0.01	**<0.01**
$PETCO_2$ (mm Hg)	39±2	38±2	26±3	38±1	25±2	37±2	38±2	22±4	35±5	39±2	23±3	<0.01	<0.01	**<0.01**
$PaCO_2$ (mm Hg)	41±2	39±2	29±2	39±2	27±3	38±3	37±3	25±2	33±4	37±4	24±2	<0.01	<0.01	**<0.01**
pH	7.41±0.02	7.44±0.02	7.46±0.02	7.43±0.02	7.47±0.03	7.44±0.03	7.44±0.03	7.49±0.03	7.45±0.05	7.42±0.02	7.45±0.02	0.01	<0.01	**0.04**
$[HCO_3^-]$ (meq l^{-1})	26±1.0	26±0.7	20±1.1	25±1.2	19±1.5	25±0.9	25±1.2	19±1.2	22±0.6	24±0.9	17±1.3	<0.01	<0.01	**<0.01**
$PETO_2$ (mm Hg)	94±3	50±1	51±3	94±3	53±4	95±3	50±0	57±6	98±6	49±1	56±5	0.36	<0.01	**0.02**
PaO_2 (mm Hg)	91±5	47±3	53±4	93±4	53±3	96±8	49±5	54±4	99±8	43±4	54±5	0.22	<0.01	**0.04**
SaO_2 (%)	98±0	85±3	87±3	98±0	87±3	98±1	85±4	87±3	98±1	82±4	89±2	0.17	<0.01	0.22
CaO_2 (ml dl^{-1})	19.2±0.5	16.8±0.8	19.7±0.8	20.2±1.0	20.0±0.7	20.7±0.6	17.9±0.6	20.8±0.7	22.1±0.6	18.0±1.0	22.0±0.5	<0.01	<0.01	**<0.01**
$\dot{V}O_2$ (ml kg min^{-1})	8±2		11±2			18±3		22±3	10±3		15±4	<0.01	<0.01	0.57

Glu (mmol l^{-1})	5.1±0.2	5.1±0.2	5.0±0.6	5.1±0.4	5.1±0.7	5.1±0.4	5.2±0.3	5.3±0.7	5.3±0.4	5.3±0.3	5.6±0.7	0.03	0.75	0.59
La (mmol l^{-1})	0.7±0.2	0.7±0.2	0.7±0.1	0.7±0.2	0.8±0.1	0.8±0.1	0.9±0.2	1.1±0.2	1.1±0.4	0.8±0.2	1.9±1.0	<0.01	0.01	**<0.01**
Cerebrovascular														
$\dot{Q}_{ICA}$ (ml min^{-1}) [a]	321±61	330±64	286±54	294±45	275±35	310±56	357±90	272±55	213±42	300±42	203±49	<0.01	<0.01	0.10
$\dot{Q}_{VA}$ (ml min^{-1}) [a]	91±28	96±30	73±31	77±23	65±24	92±25	106±42	65±30	73±16	98±39	56±19	0.06	<0.01	0.22
$\dot{Q}_{ECA}$ (ml min^{-1})	135±53	145±57	143±78	182±76	244±141	325±176	342±174	353±158	96±31	142±36	82±22	<0.01	<0.01	0.10

Table 1. Continued

	Baseline			**+0.5 ºC**		**+1.5 ºC**			**-1.0 ºC**			**Main effect**		**Interaction**
	NX	AHX	CHX	NX	CHX	NX	AHX	CHX	NX	AHX	CHX	Thermal	Hypoxia	
Haematological														
mOsm (mmol kg^{-1})	286±2	287±2	283±2	288±1	285±3	290±2	292±3	286±3	290±3	290±2	287±2	<0.01	<0.01	0.54
Hct (%)	44±1	44±1	50±1	46±2	51±1	47±1	47±2	53±1	50±1	49±2	55±2	<0.01	<0.01	0.11
[Hb] (g dl^{-1})	14.3±0.4	14.4±0.4	16.4±0.5	15.0±0.5	16.7±0.5	15.4±0.5	15.4±0.6	17.4±0.2	16.3±0.4	16.0±0.6	18.0±0.5	<0.01	<0.01	0.09
Viscosity (cP)	3.8±0.2		4.5±0.2	4.2±0.4	4.5±0.3	4.4±0.4		4.9±0.2	5.0±0.5		5.6±0.4	<0.01	<0.01	0.54
Perceptions														
Sensation (1 – 13)	7.0±0.4		6.5±0.4	9.2±0.8	9.3±0.6	10.5±0.5		11.2±0.7	3.1±0.6		2.9±1.2	<0.01	0.95	**0.04**
Discomfort (1 – 9)	1.1±0.3		1.5±0.7	4.0±1.7	3.1±1.5	6.0±1.2		6.8±2.1	6.4±1.8		6.0±1.8	<0.01	0.01	0.69
Feelings (-5 - +5)	2±2		1±2	1±1	1±2	-1±1		-2±2	-2±2		-1±2	<0.01	0.43	0.69
Cognition														
RT (ms)	378±45		361±41	380±58	359±37	335±20		340±29	416±51		393±65	<0.01	0.90	0.80
$PAPA_{ART}$ (ms)	610±85		584±44	594±69	546±42	541±62		528±69	594±50		592±74	0.02	0.10	0.20
$PAPA_{ART}$ - P_{ART} (ms)	245±61		207±48	226±48	194±49	213±70		192±83	200±32		218±65	0.44	0.20	0.27

Abbreviations: NX = normoxia, AHX = acute hypoxia, CHX = chronic hypoxia (high altitude testing). Data are expressed as mean ± SD. Main and interactive effects are presented. [a]N=8 for NX and n=7 for AHX for these variables at the -1.0 °C stage; n=8 for AHX at the +1.5 °C stage

Cerebral blood flow and oxygen delivery

The global and regional changes in blood flow are summarized in Figure 3 and Table 1. Core heating by 1.5 ^{o}C did not reliably reduce CBF at sea level (-0.5%) or high altitude (-7%; Heat main effect, p=0.98), whereas core cooling by a lesser extent (i.e. 1.0 ^{o}C) decreased CBF by 28% and 20% at sea level and high altitude, respectively (Cold main effect, p<0.01; Figure 3). Heating also did not affect CDO_2 (Heat main effect, p=0.08). Core cooling reduced CDO_2, due entirely to the reduction in CBF (Cold main effect, p=0.01). Acute hypoxia increased CBF by 4%, which was enough to maintain CDO_2 at normoxic values (Acute hypoxia CBF main effect, p<0.01; CDO_2 main effect, p=0.94). Acclimatization to hypoxia, however, caused a slight reduction in CDO_2 despite haemoconcentration having completely restored CaO_2; thus, the reduction in CDO_2 was the consequence of a 10% reduction in CBF (High altitude CDO_2 main effect, p=0.04; CBF main effect, p=0.03). Figure 4 depicts CBF and CDO_2 across all thermal and hypoxic stages.

[Figure 3]

[Figure 4]

Mechanisms of CBF regulation

Core cooling decreased CVC by 29 – 37%, while core heating increased CVC by 9 – 15% (Heat/Cold main effects, each p<0.01). At high altitude, CVC was ~13% lower regardless of thermal stress (High altitude, p<0.01; Figure 5).

[Figure 5]

At sea level, cold stress increased $CVRO_2$ (Cold main effect, p<0.01; Figure 6). This cold-induced elevation in $CVRO_2$ is the same when expressed as a function of CaO_2 (p<0.01). Heat stress tended to increase $CVRO_2$, however, this did not reach statistical significance (Heat main effect, p=0.08).

[Figure 6]

The CBF response to controlled hypocapnia while normothermic, as well as the CBF response to normocapnic restoration during thermal stress is shown in Figure 7. The $CVRCO_2$ was enhanced with core heating (main effect, p=0.03), and tended to be greater with core cooling (main effect, p=0.05), both independent of altitude. The $CVRCO_2$ was also greater at high altitude (main effect, p<0.01), regardless of thermal strain.

[Figure 7]

We were unable to consistently clamp oesophageal temperature at +1.5 °C during acute skin cooling during heat stress. The $\bar{T}sk$ was decreased from ~38 °C to ~34 °C at both altitudes, but esophageal temperature was decreased by ~1 °C in the process. Independent of altitude, acute skin cooling decreased $\dot{Q}_{ECA}$ by 190 mL min-1 [109, 271] (main effect, p<0.01) with no change in $\dot{Q}_{ICA}$ (p=0.80). These findings will be highlighted below (see *Does extracranial circulation 'steal' from the brain?*).

Functional outcomes of combined thermal and hypoxic stress (Table 1)

Effects of thermal stress on perceived body temperature were dependent on altitude (Thermal – High altitude interaction, p=0.04). Thermal discomfort was similarly affected by hypoxia regardless of the type of thermal stress (High altitude main effect, p=0.01), with participants feeling more thermally uncomfortable at high altitude.

Core heating decreased and core cooling increased reaction time, both by an average of ~8%, as assessed by Pro-trial reaction time (Heat and cold main effects each, p<0.01). Altitude did not significantly influence mean reaction time (High altitude main effect, p=0.90). Complex visuomotor reaction time and cognitive control (corrected for Pro-point reaction time), as assessed by combined pro-point/anti-point tasks, respectively, was not influenced by hypoxia or either thermal stress relative to baseline core temperature (Hypoxia main effect, p=0.20; Thermal main effect, p=0.44; Interaction effect, p=0.27).

Discussion

This study is the first to determine how CBF and CDO_2 are regulated by isolated and combined thermal and hypoxic stressors. The main findings were: (1) mild (-1 °C) core cooling decreased CBF and CDO_2 by ~25% and ~15% at both altitudes, whereas a greater extent of core heating (+1.5 °C) did not reliably decrease either CBF or CDO_2, (2) increases in CBF ensured CDO_2 was maintained when acute hypoxia was imposed during both cold and heat stress, (3) acclimatization to high altitude restored CaO_2 but transient reductions in CBF with concurrent cold stress are reflected in a lower CDO_2, and (4) combined thermal and hypoxic stress did not impair indices of cognitive function.

Together, these findings highlight that only core cooling substantially reduces CDO_2, and that altered cerebrovascular and metabolic responses might protect the brain from obvious cognitive impairment during combined cold and hypoxic stress. The following discussion considers the primary factors contributing to the regulation of CBF and CDO_2 with isolated thermal stress, then with combined thermal and acute hypoxic stress, and finally with thermal stress during acclimatization to hypoxia.

Factors contributing to CDO_2 during isolated thermal strain (Hypothesis 1)

In the context of the current findings, as outlined below, the regulation of CDO_2 under isolated thermal strain depends primarily on four factors: (1) ventilatory sensitivity to changes in core temperature, (2) the magnitude of haemoconcentration elicited by the thermal stress, (3) $CVRCO_2$, and (4) $CMRO_2$.

(1) Ventilatory sensitivity to changes in core temperature: The ventilatory response to prolonged core cooling has not been clearly characterized; however, brief bouts of cold exposure, i.e. the cold shock response, triggers hyperventilation and cerebral hypoperfusion when resting (Cooper *et al.*, 1976; Mantoni *et al.*, 2008). Cold-induced reductions in CBF are largely prevented when arterial CO_2 is kept from decreasing (Mantoni *et al.*, 2008). In alignment with our first hypothesis, the three-fold increase in $\dot{V}_E$ with core cooling contributed to a 233 mL min^{-1} decrease in CBF and 15% reduction in CDO_2. Restoring $PaCO_2$ recovered 58% of the CBF deficit and the entirety of the CDO_2 deficit generated by core cooling. These data indicate that hypocapnia-mediated cerebral hypoperfusion contributes entirely to the observed 15% decrease in CDO_2 with core cooling.

The (hyper)ventilatory responsiveness to heat stress influences CDO_2 primarily by inducing arterial hypocapnia that causes vasoconstriction and cerebral hypoperfusion (Brothers *et al.*, 2009; Nelson *et al.*, 2011). Passive heating by ~1.3 ^{o}C generally elicits a hyperventilatory response that reduces $PaCO_2$ (Fujii *et al.*, 2008; Tsuji *et al.*, 2012), but the threshold is highly variable between people [reviewed in (Tsuji *et al.*, 2016)]. This hyperthermia-induced hypocapnia is responsible for 50 – 100% of the decrease in CBF with core heating above ~38.5 ^{o}C (Brothers *et al.*, 2009; Nelson *et al.*, 2011; Bain *et al.*, 2013; Tsuji *et al.*, 2015, 2018, 2019). Countrary to our first hypothesis, because of the generally modest ventilatory response in our participants, core heating by 1.5 ^{o}C did not decrease CBF. With maintained cerebral autoregulation (Low *et al.*, 2009), enhanced $\dot{Q}$ [and its potential implications on CBF (van Lieshout *et al.*, 2001; Ogoh *et al.*, 2005)], and presumably increased $CMRO_2$, it seems that the vasoconstrictor stimuli afforded by the small 3 mm Hg drop in $PaCO_2$ with core heating was not enough to reliably decrease CBF.

(2) Magnitude of haemoconcentration: Haemoconcentration occurs acutely with cooling and heating, and impacts CDO_2 by increasing [Hb] (i.e., see Eq: 5). In the present study, haemoconcentration (+15% [Hb]) occurred with core cooling, which was reflected in a 15% increase in CaO_2 and consequently a substantial effect on CDO_2. Although these changes might seem small they are nonetheless physiologically meaningful. For instance, in the absence of cooling-induced haemoconcentration, CDO_2 would have dropped by 26% as opposed to the observed 15%, illustrating its protective effect in maintaining CDO_2.

The 8% increase in [Hb] with core heating was responsible for the 8% increase in CaO_2 and slight increase in CDO_2 (Figure 4). Haemoconcentration during heat stress is sometimes interpreted to indicate some level of dehydration, which has been shown to potentiate reductions in CBF and CDO_2 (Trangmar *et al.*, 2014, 2015). Additionally, the haemoconcentration that occurred with both cooling and heating caused an increase in blood viscosity (Table 1), which would be expected to compromise CBF according to Poiseuille's Law (Nichols *et al.*, 1974). However, the influence of blood viscosity on CBF is likely negligible in comparison to the effect of haemoconcentration in stimulating oxygen-sensing mechanisms in the brain, as has been shown in studies that modulate viscosity without altering CaO_2 (Grotta *et al.*, 1982; Brown & Marshall, 1985; Tomiyama *et al.*, 2000).

(3) Cerebrovascular reactivity to changes in $PaCO_2$: This reactivity will determine the magnitude of cerebral hypoperfusion with thermally-mediated arterial hypocapnia. The present findings support that heat stress increases $CVRCO_2$ irrespective of altitude (Figure 7). Although counter to previous findings that show heating does not affect (Low *et al.*, 2008; Lee *et al.*, 2015) or slightly decreases (Lee *et al.*, 2014) $CVRCO_2$, this is the first investigation (to our knowledge) to directly compare normothermic and heated $CVRCO_2$ using volumetric measures of CBF from all arteries. Moreover, we investigated $CVRCO_2$ solely within the same hypocapnic range between 20 – 40 mm Hg to ensure linearity between CBF and $PaCO_2$ and direct comparisons for all stressors. This necessitated a relative step-down when normothermic and a relative step-up when heat stressed, as can be visualized in Figure 7. Mechanistically, the increased $CVRCO_2$ with heat stress might be accounted for by increased MAP sensitivity to CO_2 perturbations with heat stress at high altitude. Indeed, MAP increased nearly 6 times more when heating-induced hypocapnia was returned to normothermic values (Heat – Chronic Hypoxia MAP/$PaCO_2$ interaction, $p<0.01$). This increased MAP sensitivity has been reported previously with acclimatization to high altitude (Fan *et al.*, 2014, 2016; Willie *et al.*, 2015). These findings provide evidence that heat stress augments this effect.

(4) $CMRO_2$: The $CMRO_2$ will impact CDO_2 primarily through its influence on CBF due to the tight regional and temporal coupling of neural activity and blood flow [reviewed in (Phillips *et al.*, 2016)].

This coupling between regional CBF and metabolism allows for regulation of local cerebral perfusion *and* temperature (Yablonskiy *et al.*, 2000). Temperature affects $CMRO_2$ in proportion to its Q_{10} coefficient, which characterizes the rate of a reaction as a function of changing temperature. Existing data on the Q_{10} coefficient of cerebral tissue is sparse and inconsistent, so assigning the role of $CMRO_2$ in the regulation of CDO_2 is challenging (Stone *et al.*, 1956; Greeley *et al.*, 1993; Nybo *et al.*, 2002*a*). However, the role of $CMRO_2$ might be elucidated by the large decrease in CBF with cold stress, of which only 58% can be explained by arterial hypocapnia. With core cooling, $\dot{Q}$ and MAP are elevated, both of which would be expected to increase CBF. Therefore, it seems reasonable to speculate that the remainder of the decrease in CBF with core cooling would be a consequence of either sympathetic vasoconstriction of the cerebrovasculature (Faraci *et al.*, 1987; Cassaglia *et al.*, 2008) or decreased $CMRO_2$ (Stone *et al.*, 1956; Greeley *et al.*, 1993).

Combined thermal and acute hypoxic stress and interactions on CBF and CDO_2 (Hypothesis 2)

In relation to the four factors that contribute to the regulation of CDO_2 during thermal stress (discussed above), acute hypoxia will largely only contribute to CBF and CDO_2 through its vasodilatory influence on the cerebrovasculature [reviewed in (Hoiland *et al.*, 2016)]. Haematological adjustments to hypoxia require days (Lucas *et al.*, 2011) and are therefore absent in the acute hypoxic setting. The combined influence of thermal and acute hypoxic stress on $CMRO_2$ is unknown, but $CMRO_2$ appears to be unaltered in acute hypoxia *per se* (Ainslie *et al.*, 2014). In the present study, the cerebral vasoconstrictor stimuli afforded by cold and heat stress was completely overcome by acute hypoxia (Figure 6). The hypothermic-induced cerebral vasoconstriction was substantial, i.e. CVC decreased by 37% (Figure 5). With cooling, however, there was an 8-fold increase in $CVRO_2$ that returned CBF back to normothermic values, and nearly fully restored the 15% decrease in CDO_2, which is in opposition to our second hypothesis. The dramatic increase in CBF with combined cold and acute hypoxic stress was likely mediated partly by the 4-mm Hg increase in $PaCO_2$ (Table 1). As arterial blood gases were matched to those attained during normothermic poikilocapnic hypoxia, the arterial hypocapnia induced during core cooling was partly restored (from 33 to 37 mm Hg). The cerebrovascular reactivity to CO_2 with core cooling was 22 mL min^{-1} mm Hg^{-1} at sea level; therefore the 4-mm Hg increase in $PaCO_2$ with isocapnic hypoxia would be expected to contribute ~89 ml min^{-1} of the observed 238 ml min^{-1} increase in CBF (~37%). Despite this being a meaningful contribution to the blood flow response, the hypoxia-mediated increase in CBF is still 4 to 5 times greater when cold compared to thermoneutral.

With core heating, the hypocapnia and associated cerebral hypoperfusion was minimal, and acute hypoxia caused a net vasodilation, resulting in a 12% increase in CBF and 28% increase in CVC

relative to normothermic normoxia. Again, counter to our second hypothesis, this increase in CBF coupled with heat-induced haemoconcentration maintained CDO_2 despite a 7% reduction in CaO_2 when $P_{ET}O_2$ was clamped at 50 mm Hg.

Combined thermal and chronic hypoxic stress and interactions on CBF and CDO_2 (Hypothesis 3)

Acclimatization to hypoxia comes with a myriad of ventilatory, haematological and autonomic adaptations that act in coordination to maintain CDO_2 at sea level values in the face of decreased atmospheric oxygen content [reviewed in (Hoiland *et al.*, 2018) and also evident in Table 1]. Of the four primary factors contributing to CDO_2 with thermal stress, nearly all will be influenced by the adaptations occurring with acclimatization to high altitude. These four factors are outlined in the context of the current findings at high altitude.

(1) Thermal stress, ventilatory acclimatization and acid-base balance at high altitude: After 16 days at 4330 m, CBF and CDO_2 were nearly returned to sea-level values. These changes occurred despite the marked hypoxaemia and arterial hypocapnia and are largely explained by the influence of metabolic compensation via haemoconcentration and respiratory alkalosis (Howe *et al.*, 2019). Despite this restoration of CBF and CaO_2 (and hence CDO_2) at high altitude, concurrent thermal stress gave rise to a reduction in both CBF and CDO_2. These reductions in CBF and CDO_2 seem to be due to an augmented thermally-mediated hyperventilatory response at high altitude, indicating a synergistic effect between stressors (Table 1). To the best of our knowledge, there are no existing data on ventilatory responses to thermal stress during acclimatization to high altitude. However, active (Chu *et al.*, 2007) and passive (Petersen & Vejby-Christensen, 1977) heating have previously been shown to augment the *acute* hypoxic ventilatory response. The relatively greater increase in $\dot{V}_E$ with heat and acute hypoxia compared to heat and chronic hypoxia is likely due in part to the background of hypocapnia present during ventilatory adaptation to high altitude as the prevailing circulating CO_2 plays a critical role in the isocapnic hypoxic ventilatory response (Ainslie & Poulin, 2004; Duffin, 2007). The interactive influence of heating (McQueen & Eyzaguirre, 1974) and hypoxia (Lahiri & DeLaney, 1975) might be mediated via increased afferent nerve activity from the carotid body; a response that is likely further sensitized during chronic hypoxia exposure (Arias-Stella & Valcarcel, 1976; Wang & Bisgard, 2002; Wang *et al.*, 2008). This explanation likely does not explain the interaction between cooling and hypoxia, as directly cooling the carotid body decreases carotid sinus nerve activity (McQueen & Eyzaguirre, 1974); however, muscle contraction with light exercise has been shown to sensitize the peripheral chemoreflex response (Weill *et al.*, 1972). It seems possible that muscle contraction during shivering might cause a similar interaction.

In the context of CDO_2 regulation, core cooling and heating to the same magnitude as at sea level caused greater increases in $\dot{V}_E$ but these did not correspond to proportionally greater reductions in $PaCO_2$ (Table 1), the actual stimuli for cerebral vasoconstriction. This disconnect in $\dot{V}_E$ and $PaCO_2$ with changes in core temperature at high altitude does not appear to be explained by disproportionate increases in f_B or enhanced $P_{ET}CO_2$-$PaCO_2$ concentration gradient (Kronenberg *et al.*, 1971; Tymko *et al.*, 2015). Indeed, alterations in metabolism, acid-base balance (i.e., HCO_3^-) and/or an influence of temperature on ventilation-perfusion matching may partly explain these changes.

(2) Thermal stress and haemoconcentation at high altitude: Haemoconcentration occurs independently with cooling, heating and prolonged hypoxic exposure, and the interaction between thermal and hypoxic stress appears to be additive for effects on [Hb], Hct and viscosity (Table 1). In agreement with our third hypothesis, concurrent increases in $\dot{V}_E$ and [Hb] completely restored CaO_2 to sea level values (Table 1). The 15% increase in Hb is substantial and contributes almost entirely to the 18% difference in CaO_2 between acute hypoxia at sea level and that observed at high altitude. Core cooling at high altitude elicited the highest [Hb] and nearly highest CaO_2 in the entire investigation, yet CDO_2 was decreased by 19% due to a 29% decrease in CBF, again in alignment with our third hypothesis. Haemoconcentration with core cooling is likely consequent to the combined influence of cold-induced diuresis [reviewed in (Pozos & Danzl, 2014)], splenic contraction (Kanter, 1968; Bakovic *et al.*, 2005) and plasma leakage from the vascular space (Wolf *et al.*, 1992), and provides novel insight into the interactive regulation of CBF and CDO_2 in the context of chronic hypoxia. Contrary to our third hypothesis, however, core heating at high altitude provided additional haemoconcentration and further increased CaO_2; together, this was enough to maintain CDO_2 despite the 7% decrease in CBF.

(3) $CVRCO_2$ and high altitude: The $CVRCO_2$ increased with chronic hypoxic exposure, which is consistent with some previous reports (Fan *et al.*, 2010, 2014; Lucas *et al.*, 2011; Flück *et al.*, 2015; Willie *et al.*, 2015). This is largely explained by the combined effects of reduced hydrogen buffering capacity and increased cerebral perfusion pressure (Fan *et al.*, 2014, 2016; Willie *et al.*, 2015). For example, in the current study, the HCO_3^- was reduced from 26 to 20 meq L^{-1} with acclimatization, so that a given change in $PaCO_2$ would correspond with a greater change in cerebrospinal fluid pH (and hence stimulus on $CVRCO_2$). Indeed, when CBF is expressed a function pH or H+, the observed increase in CO_2 reactivity is no longer evident (pH and H+ both, $p>0.65$). Additionally, MAP sensitivity to $PetCO_2$ perturbations was enhanced at high altitude ($p<0.01$; data not shown), similar to that reported previously at slightly higher altitudes (Fan *et al.*, 2014, 2016; Willie *et al.*, 2015). Despite this increased $CVRCO_2$, the cerebrovascular response to thermal stress at high altitude was

comparable to that observed at sea level, i.e., the magnitude of cerebral hypoperfusion was largely dependent on thermally-mediated hypocapnia. Finally, it was noteworthy that over the range of manipulations in core temperature, CVC was decreased at high altitude. It seems plausible that the observed decrease in CVC at high altitude in the present study is completely accounted for by the slight increase in CaO_2 and profound arterial hypocapnia, resulting in a 13% decrease in CBF. Indeed, it is difficult to ascribe changes in cerebrovascular tone as responses to changes in blood pressure when SaO_2, CaO_2 and $PaCO_2$ are dramatically changing while MAP remains stable (Table 1).

(4) $CMRO_2$ and high altitude: It has been shown that $CMRO_2$ is stable at altitudes up to 5000 m owing to the tight regulation of CDO_2 (Severinghaus *et al.*, 1966; Møller *et al.*, 2002; Willie *et al.*, 2015), and any interactive influence of thermal stress is presently unknown.

[Figure 8 – Revised]

Implications of thermal and hypoxic stress on cognitive function (Hypothesis 4)

That core cooling and heating increased and decreased reaction time, respectively, could be explained by the effect of temperature on (mostly peripheral) nerve conduction velocity (Rammsayer *et al.*, 1995; Kiernan, 2001; Drenthen *et al.*, 2006). Piil and colleagues (2017) recently showed that the negative impact of hyperthermia was exposed only when task complexity was maximized (Piil *et al.*, 2017). Although the pro/anti-point task (which combines all of the tasks, i.e. reaction time, inhibitory control and cognitive control) was the most cognitively demanding task in our battery, it is not as multifactorial as the testing battery used by Piil and colleagues, so the absence of a deterioration in pro/anti-point reaction could be expected. That hypoxia did not reliably influence any index of cognition is perhaps not surprising given the variation in neurocognitive data presented over the last 15 years (Virués-Ortega *et al.*, 2004; Maiti *et al.*, 2008; Turner *et al.*, 2015; McMorris *et al.*, 2017; Nakata *et al.*, 2017; Caldwell *et al.*, 2018; Hüfner *et al.*, 2018). This variability, while perhaps physiological in part, is undoubtedly related to widespread variability in hypoxic stimuli and the vast diversity of cognitive batteries used. It is interesting that thermal stress at high altitude did not incur any observable cognitive deficit despite reductions in CDO_2. Given the proposed influence of cerebral lactate metabolism on cognition (Tsukamoto *et al.*, 2016; Hashimoto *et al.*, 2018), the increase in cerebral lactate delivery with thermal stress at high altitude may explain the maintenance of cognitive performance in the face of reduced CDO_2. Indeed, cerebral lactate delivery was increased 47% with heating and 100% with cooling at high altitude.

Does extracranial circulation 'steal' from the brain?

It has been suggested that changes in extracranial vascular conductance may influence CBF by virtue of redirecting blood flow from the ICA to the ECA (Ogoh *et al.*, 2013, 2014; Sato *et al.*, 2016). Inducing acute hypoxia with core temperature displacements in both directions provides unique insight into this question. Core heating at sea level increased ECA conductance by 173%, while $\dot{Q}_{ICA}$ was only decreased by only 3% (Table 1). With the imposition of acute hypoxia during heat stress, $\dot{Q}_{ICA}$ increased by 13% with no change in $\dot{Q}_{ECA}$, illustrating appropriate CBF regulation with near maximal facial/scalp vascular conductance. This increase in CBF with acute hypoxia during heat stress (+120 mL min^{-1}) could have been accommodated by the ~100 mL min^{-1} increase in $\dot{Q}$ (Figure 8). Moreover, when mean skin temperature was acutely decreased by 4 ºC with elevated core temperature, $\dot{Q}_{ECA}$ was decreased by 50 – 70% ($p<0.01$) with no change in $\dot{Q}_{ICA}$ ($p=0.80$), indicating the high facial/scalp conductance was not 'stealing' blood from the cerebral circulation during the experimental conditions of the current study [which may differ in exercising heat stress when $\dot{Q}$ is maximized (Sato *et al.*, 2016; Chou *et al.*, 2018)]. Core cooling on the other hand, resulted in a proportionally smaller decrease in ECA conductance compared to heat stress, which is in support of previous reports suggesting the face/scalp circulation has limited capacity to constrict (Froese & Burton, 1957). Acute hypoxia during core cooling resulted in proportionally similar increases in both $\dot{Q}_{ICA}$ (+38%) and $\dot{Q}_{ECA}$ (+45%), indicating that the extracranial circulation may passively accommodate large increases in common carotid artery blood flow during cerebral vasodilation.

Experimental limitations

The primary limitation of this investigation is sample size. Time constraints and participant availability/willingness limited this study to 12 healthy male participants, which led to 8 cold and 9 heat exposures at both sea level and high altitude. The availablity of only males as participants is unfortunate, but differences in CBF regulation during thermal and hypoxic stress have not been reported and therefore we do not see this as a major limitation given that many of these findings are novel for humans. Cold and heat exposures would ideally have been completed on different days to avoid potential order effects, however, that would have exposed participants to multiple arterial cannulations over a two week period and also further constrained sample size due to time demands and limitations. We attempted to lessen potential order effects by randomizing the order of thermal exposures and incorporating order as a fixed effect in the mixed model analysis. In retrospect, it would have been interesting to induce thermal strain to +2 ºC core temperature to ensure the hyperthermia-induced hyperventilatory threshold was reached in all participants. However, it is very likely that this magnitude of heat strain would have caused considerable participant dropout when

combined with hypoxia, and given that comprehensive measurements were to be prioritized at only one hot and cold stage each, +2 ºC would have been a less commonly experienced level of heat strain, and more thermally mismatched from cold stress. In the context of extreme environments, humans are most often exposed while upright and moving. How CBF and CDO_2 are regulated during exercise in these multi-factorial environments is warranted. Lastly, having a measure of $CMRO_2$ would provide further insight into the underlying mechanisms driving CBF and CDO_2 with thermal stress. Future research should investigate how similar magnitudes of passive core cooling and heating alter $CMRO_2$ to elucidate the Q_{10} coefficient of cerebral tissue.

Perspectives

The synergistic interaction between thermal and hypoxic stress on $\dot{V}_E$ has applicability for basic science, clinical contexts and the sojourner or permanent resident of high altitude environments. For example, ventilatory reserve tested at moderate altitudes appears predictive of summit success without supplementary oxygen (Bernardi *et al.*, 2006). As cold and heat strain greatly increase $\dot{V}_E$ (and presumably reduce ventilatory reserve), managing a stable core temperature might be critical for efficient locomotion at high altitudes (Amann *et al.*, 2007; Bradbury *et al.*, 2018). Another important consideration is that the 2-fold increase in $\dot{V}_E$ with mild cooling at high altitude will double the rate of respiratory heat and water loss. Furthermore, the rate of core cooling was nearly doubled at high altitude, which indicates that protecting temperature stability might be more challenging in this extreme (cold) environment (Figure 2).

It is notable that thermal sensations, i.e. how individuals perceive their thermal state, as well as the associated thermal discomfort, were sensitized at high altitude (Table 1). Whether thermal and hypoxic stimuli have additive effects within thermosensitive tracts or nuclei, or cellular hypoxia within the medulla alters thermal perceptions, appears not to be known. Irrespective, sensitization of thermal perceptions would have practical value in helping to drive behavioural thermoregulation earlier, as this is more sensitive and powerful than autonomic thermoregulation (Schlader *et al.*, 2013), and the physiological costs of these combined stressors are magnified centrally and peripherally [Table 1; (Lloyd & Havenith, 2016)].

Finally, the extent of total body cooling in this investigation was mild, yet resulted in considerable decreases in CDO_2 at both altitudes. The high metabolic demand and negligible energy reserve within the brain necessitates a constant supply of oxygen to the brain. As such, high altitude environments pose a threat to cerebral energy balance due to the combined challenges of hypobaric hypoxia and cold ambient temperatures.

Conclusion

Alterations in CBF regulation ensured CDO_2 was maintained within the range of 130 – 172 mL min^{-1} during different combinations of moderate thermal and hypoxic stress. Core cooling resulted in the greatest decreases in CDO_2 (up to 20%) and was caused entirely by decreases in CBF. Gross indices of cognitive function were not impaired by thermal or hypoxic stress in isolation or combination, despite significant stressor interactions on $\dot{V}_E$ and thermal sensations. These findings highlight that cardiovascular, cerebrovascular and metabolic responses accommodate moderate levels of thermal and hypoxic stress so that cerebral function is not obviously compromised.

Competing interests

None to declare.

Author contributions

TDG, JDC and PNA conceived the research and designed the protocol along with MMT, KNT, LCW and MS. TDG, TGD, AP, GBC, HGC, CAH, RLH, MMT, CG and AS acquired the data. TDG, PNA, JDC, KNT, LCW and APA interpreted and analysed the data. All authors revised the manuscript and provided intellectual feedback and agree to be accountable for all aspects of the work.

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Figure Captions

Figure 1. Schematic of the experimental protocol. Blood flow was measured at the internal carotid artery, external carotid artery and vertebral artery at stages 1 (normoxia), 2 (end-tidal CO_2 manipulation) and 3 (acute hypoxia; indicated by ultrasound probe), along with arterial blood samples (indicated by blood droplet). Cognition and thermal perceptions were assessed during stage 1. Blood flow, arterial blood gases, cognition and perceptions were also measured during core heating when the subject was +0.5 ºC from baseline. This protocol was completed at sea level (344 m) and repeated after ~16 days at high altitude (4330 m) without stage 3. The order of heating and cooling was randomized between participants.

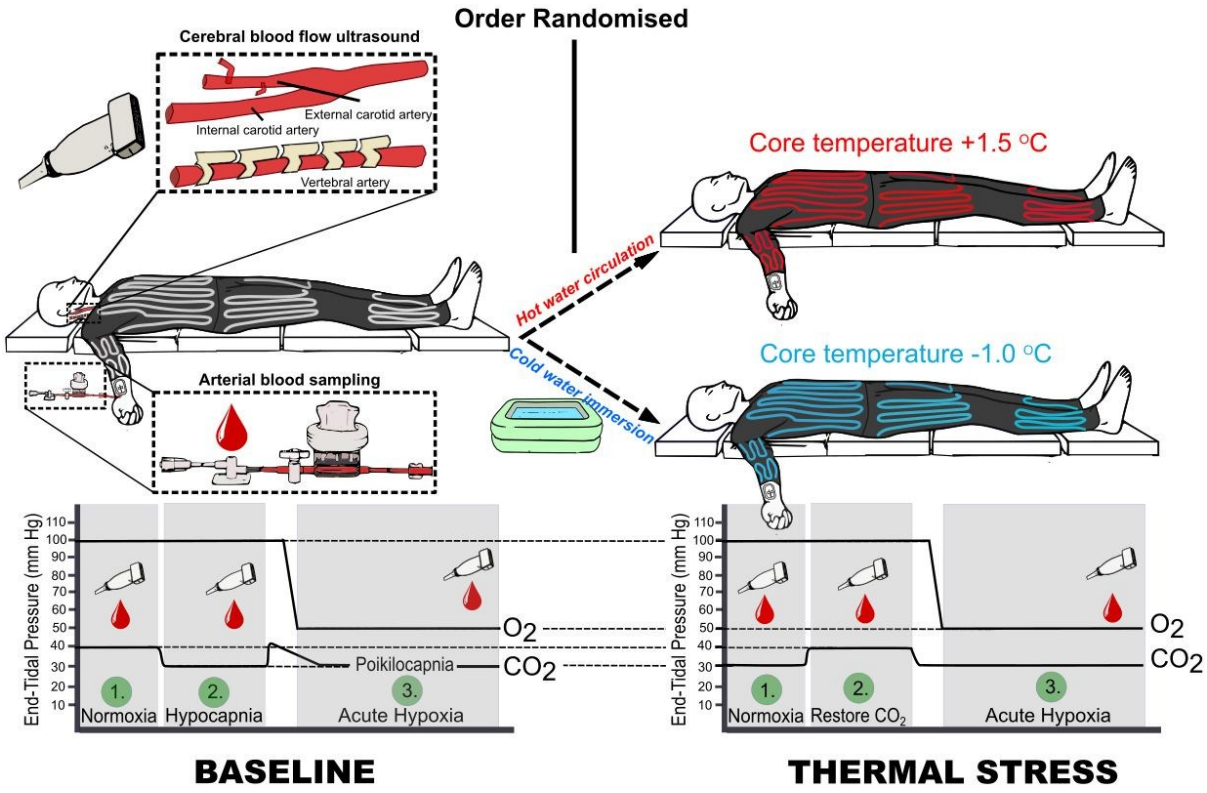


Figure 2. A. Cold immersion water temperature and water-perfusion suit (heating) temperatures at sea level (SL) and high altitude (HA), mean ± SD. B. The rate of oesophageal temperature change with heating and cooling at SL and HA.

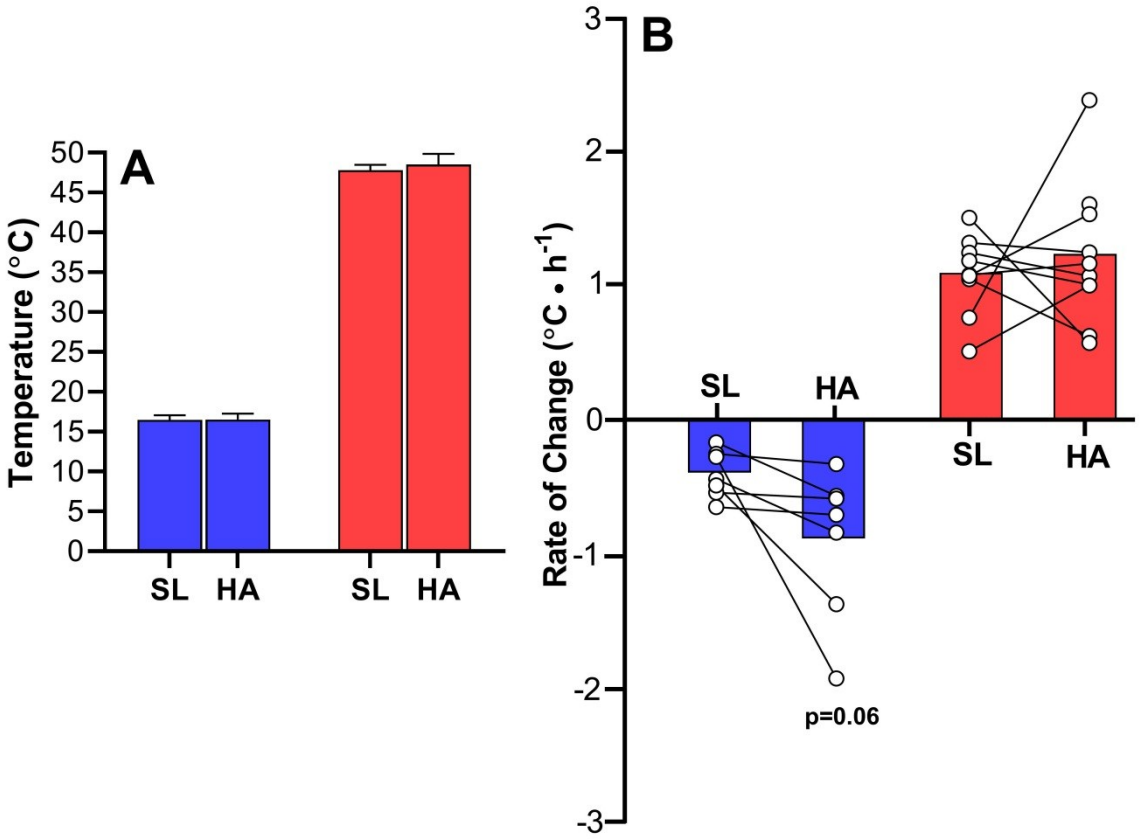


Figure 3. Cerebral blood flow (CBF) responses to cooling (-1.0 ºC) and heating (+ 1.5 ºC) during normoxia (NX; $PetO_2$ ≈ 94 mm Hg), acute hypoxia (AHX; $PetO_2$ ≈ 50 mm Hg) and chronic hypoxia (CHX; $PetO_2$ ≈ 51 mm Hg). * denotes a significant difference when compared to NX, and # denotes a difference when compared to CHX. N=8 for NX and n=7 for AHX for these variables at the Cold stage; n=8 for AHX at Hot stage.

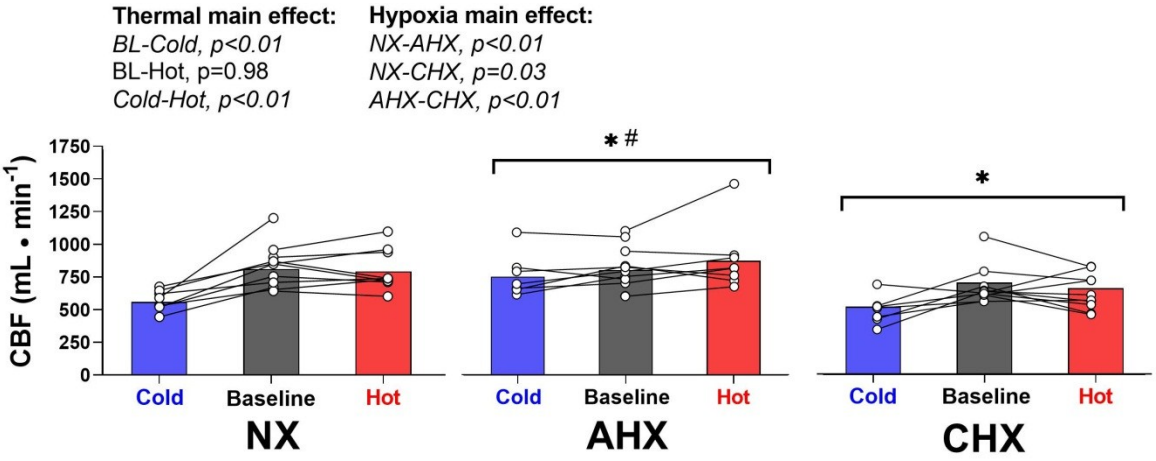


Figure 4. Cerebral blood flow (CBF; coloured bars, mean ± SD) and cerebral oxygen delivery (CDO_2; superimposed unfilled squares) across each combination of thermal and hypoxic stressors. The green horizontal line indicates resting CDO_2 at sea level. * denotes a significant difference when compared to Baseline (BL), and # denotes a significant difference when compared to Hot. NX, normoxia; AHX, acute hypoxia; CHX, chronic hypoxia. N=8 for NX and n=7 for AHX for these variables at the Cold stage; n=8 for AHX at Hot stage.

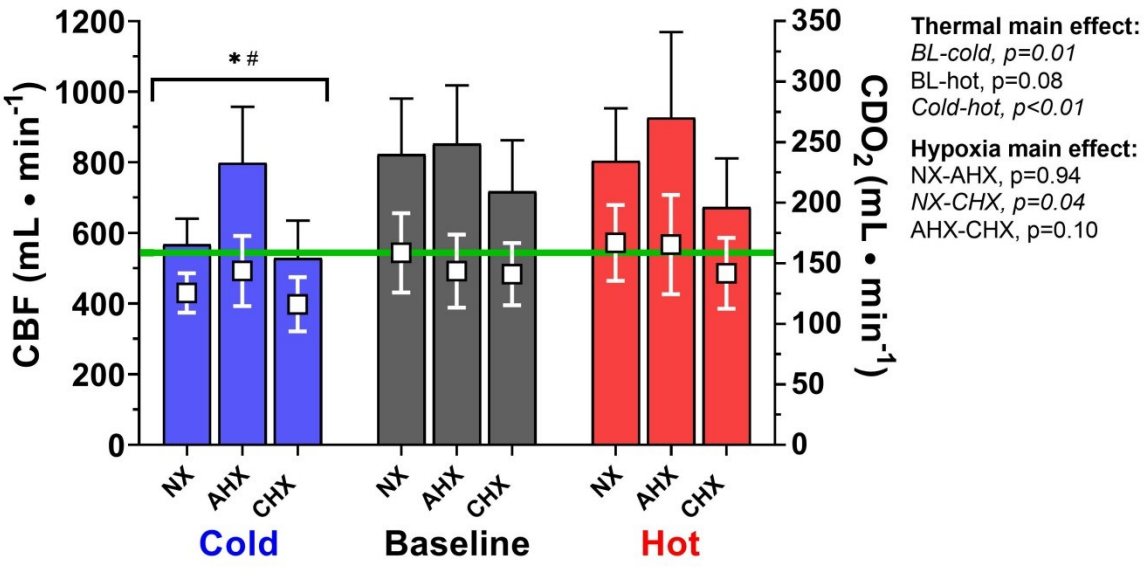


Figure 5. Cerebrovascular conductance (CVC) across changes in core temperature (ΔT_{Core}) at sea level (SL) and high altitude (HA). * represent main effect of temperature, and # represents main effect of altitude.

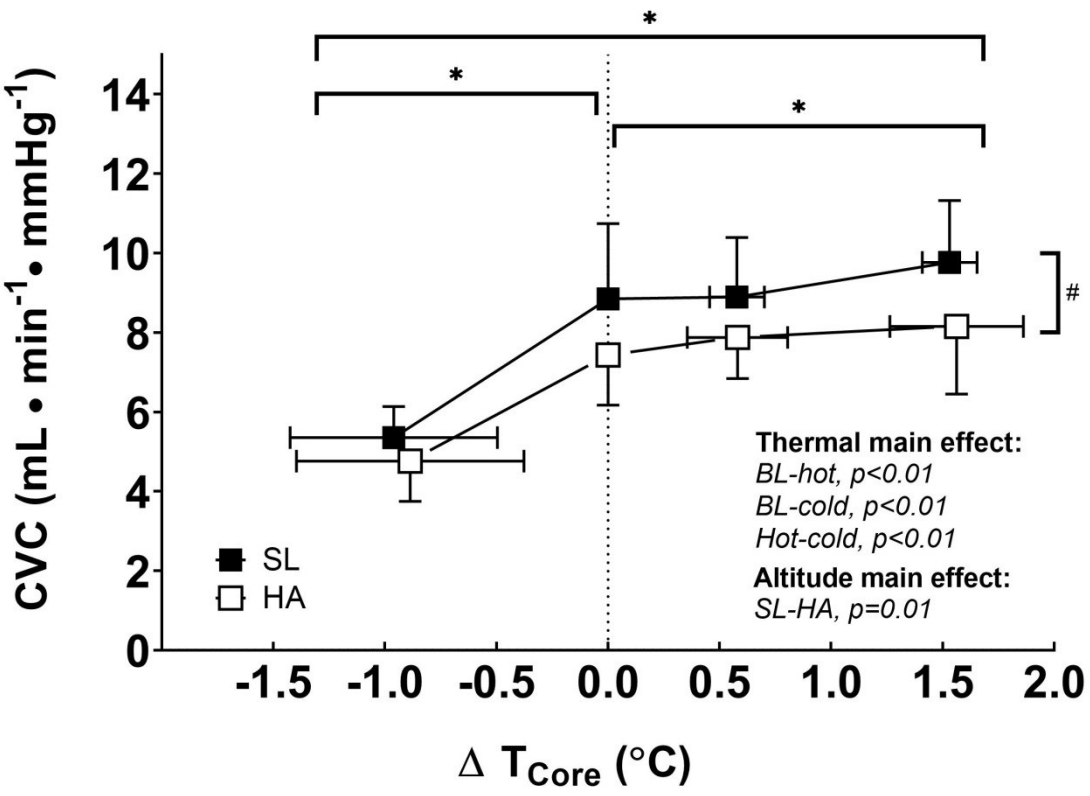


Figure 6. A. Percent change in cerebral blood flow (CBF) from normothermic baseline as a consequence of cooling and heating, and with the imposition of acute hypoxia. The individuals responses to acute hypoxia at baseline (black), cold (blue) and hot (red). SaO_2 (%) at each stage is shown in text below the lines (mean ± SD). B. The slopes of the mean responses of $CVRO_2$ from A. * denotes a significant difference when compared to Baseline, and # denotes a significant difference when compared to Hot. The hatched area represents the proportion of the $CVRO_2$ that could be accounted for by the 4 mm Hg increase in $PaCO_2$. N=8 for NX and n=7 for AHX for these variables at the Cold stage; n=8 for AHX at Hot stage.

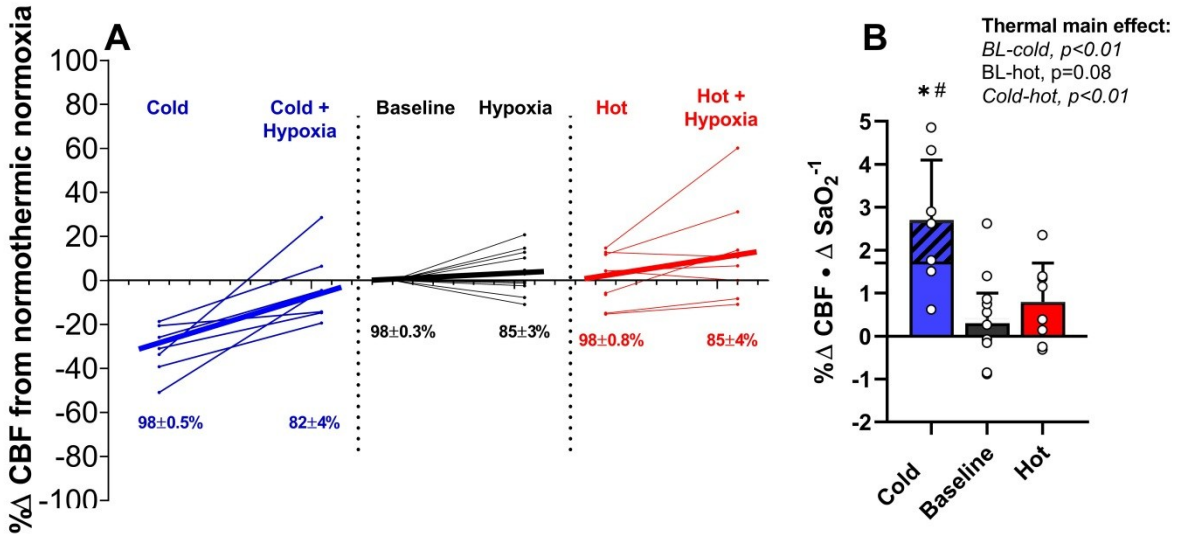


Figure 7. Percent change in cerebral blood flow (CBF) as a function of arterial CO_2 pressure ($PaCO_2$) at sea level (SL) and high altitude (HA) during acute hypocapnia at normothermic baseline and with acute CO_2 restoration during both cold and heat stress. The vertical lines (dashed at HA) represent the room air breathing poikilocapnic $PaCO_2$ at each thermal stage. * denotes a significant difference when compared to sea level. N=5 for eucapnia restoration at SL and n=7 for eucapnia restoration at HA during Cold; and n=8 for eucapnia restoration at SL and n=9 for eucapnia restoration at HA during Hot.

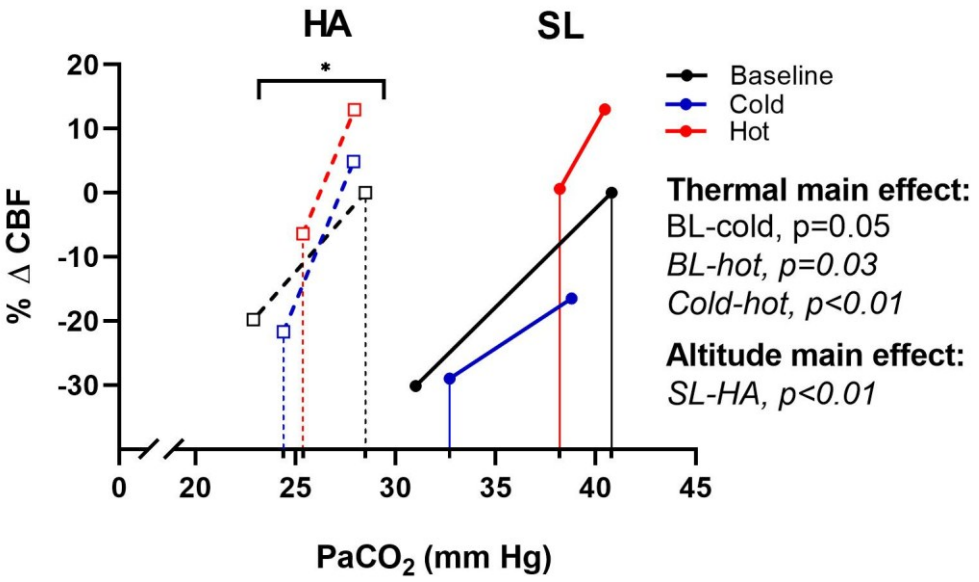
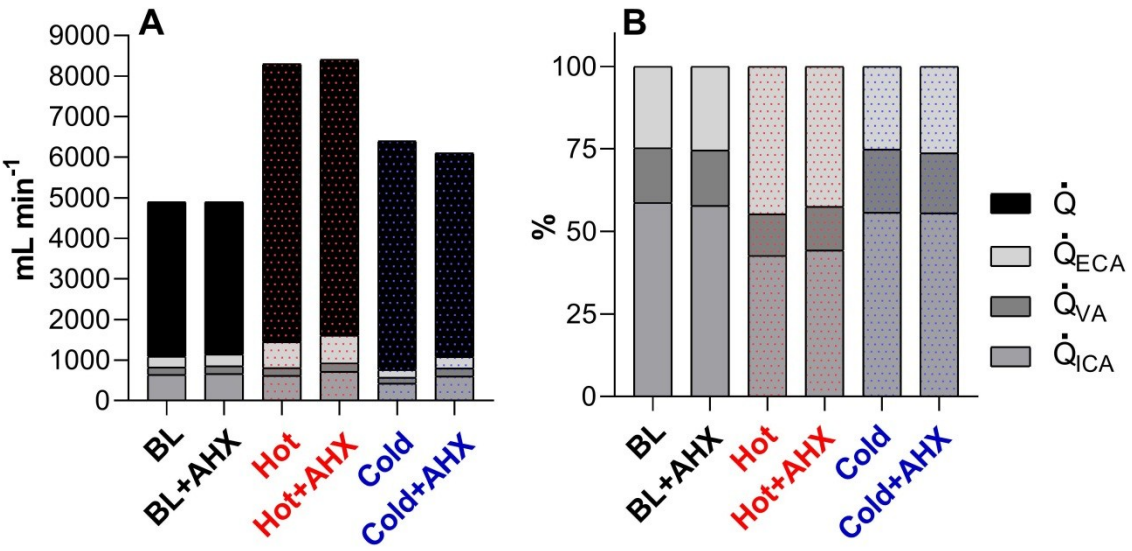


Figure 8. A. The distribution of cardiac output ($\dot{Q}$) to the external carotid (ECA), vertebral (VA) and internal carotid (ICA) at baseline (BL), with core heating (Hot) and core cooling (Cold) with and without acute hypoxia (AHX). B. The percentage distribution of blood flow through each of the conduit arteries as a proportion of total head blood flow. N=8 for NX and n=7 for AHX for these variables at the Cold stage; n=8 for AHX at Hot stage.



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